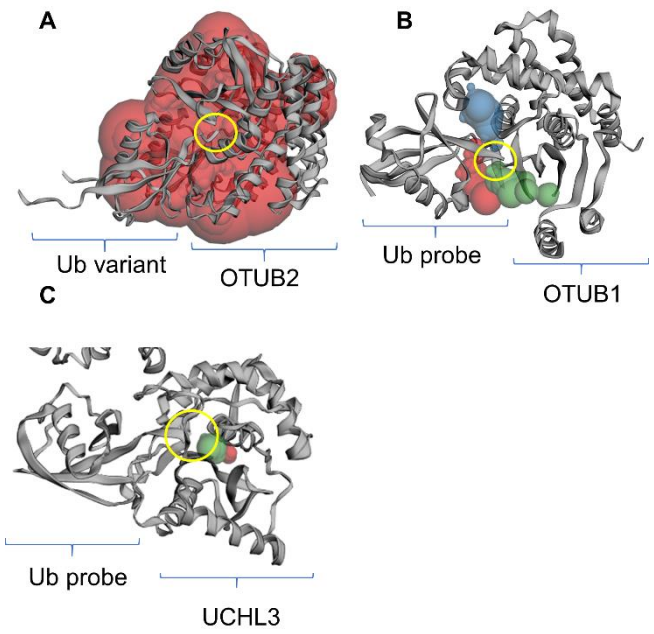
Electronic Supplementary Material (ESI) for Chemical Science.

Table of contents:

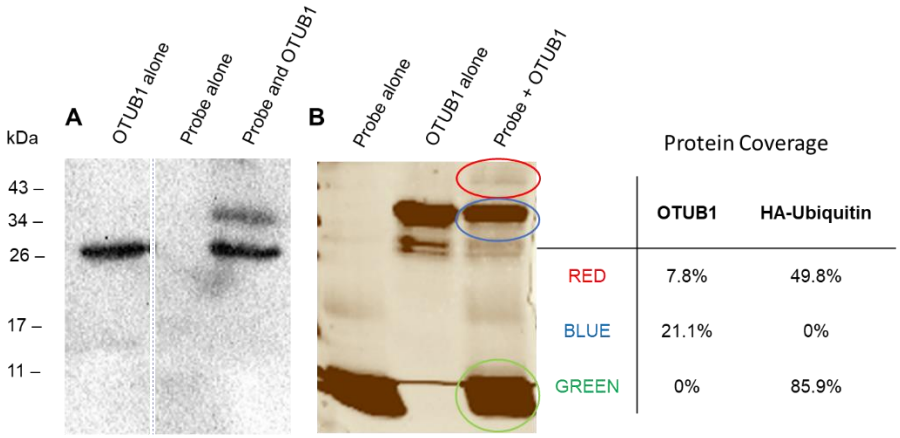
1. Supporting biological results

1.1 Visualisation of solvent accessibility



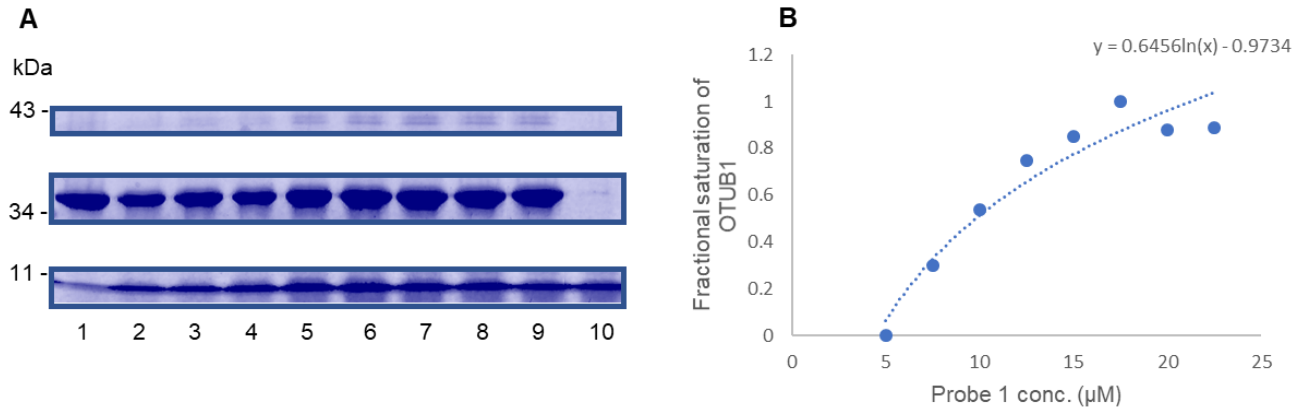
Supplementary figure 1: (A) Crystal structure of OTUB2 covalently bound to mono-Ub probe suicide probe displaying one solvent accessible pocket (red).[1] (B) Crystal structure of OTUB1 bound to a ubiquitin variant with three solvent accessible pockets highlighted (red, blue, green).[2] (C) Crystal structure of UCHL3 covalently bound to mono-Ub suicide probe displaying two solvent accessible pockets (red, green).[3] In each case the position of the Ub C-terminus is highlighted by a yellow circle. Images obtained using CASTp 3.0 software.[4]

1.2 Recombinant enzyme labelling followed by in-gel digestion



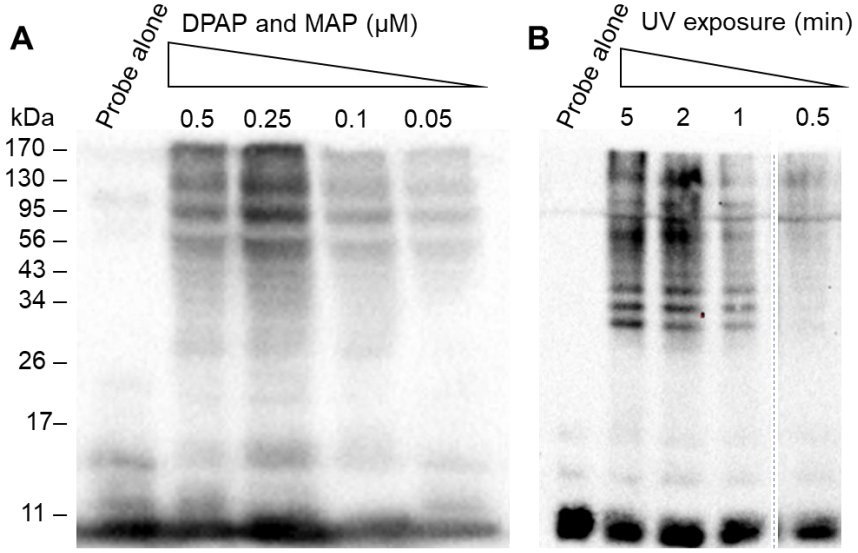
Supplementary figure 2: Labelling of recombinant DUB OTUB1 (3 µg) with probe **1** (6 µg) was visualised by (A) anti-His western blotting and (B) silver staining. Following separation by SDS-PAGE. Indicated bands on the silver stain were digested and analysed by captive spray ionisation mass spectrometry. Protein coverage refers to the % of the proteins sequence identified.

1.3 Probing the Kd of the construct with OTUB1



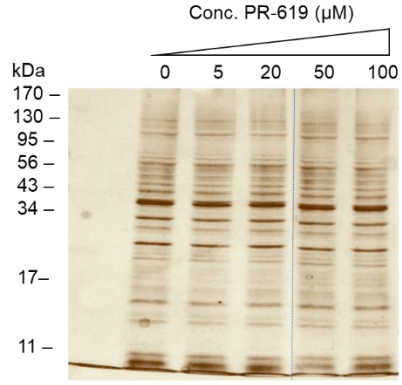
Supplementary figure 3: (A) Recombinant DUB OTUB1 (5 µg) was labelled with increasing concentrations of probe **1.** Labelling was visualised by Coomassie blue staining following separation by SDS-PAGE. (B) Intensity of the labelled band was analysed on Image Quant and plotted against the concentration of probe.

1.4 Optimisation of labelling conditions



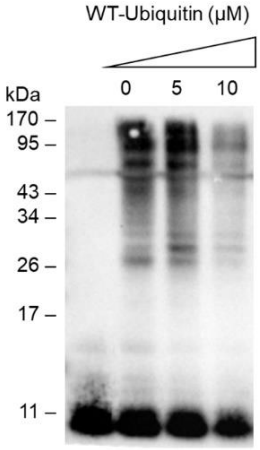
Supplementary figure 4: (A) Optimisation of initiator concentration. The probe was incubated with HEK293T lysate for 90 min before the radical initiator 2,2-dimethoxy-2-phenylacetophenone (DPAP) was added at varying concentrations along with the radical stabiliser methoxyacetophenone (MAP). Samples were degassed and exposed to UV light (365 nm) for 2 min. (B) Time under UV was investigated using DPAP and MAP (0.25 µM) to improve compatibility with LC-MS/MS. The initiators were added to the samples after a 90 min incubation and exposed to UV light for the time indicated.

1.5 Loading control for probe displacement with PR-619 assay



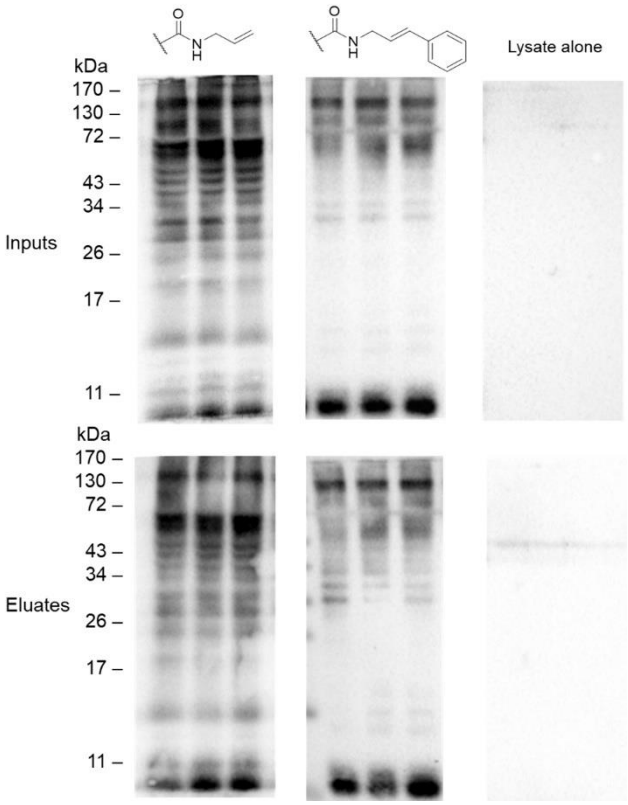
Supplementary figure 5: HEK 293T cell lysate (50 µg) was preincubated with probe **1** (1 µg) for 90 min before PR-619 was added at increasing concentrations. Samples were separated on 12% SDS-PAGE and visualised by silver staining.

1.6 Inhibition of labelling using wild-type ubiquitin



Supplementary figure 6: WT-Ub competes with the probe to bind deubiquitinating enzymes before activation with UV light, disrupting the equilibrium when added in excess. The probe (1µM) was incubated with HEK 293T cell lysate (50 µg) for 90 min. WT ubiquitin was added at increasing concentrations and incubated for 30 min before addition of radical initiators and exposure to UV light (365 nm).

1.7 Inputs and eluates from immunoprecipitation experiment



Supplementary figure 7: Three labelling reactions and immunoprecipitations were performed in parallel, in triplicate using the terminal alkene probe **1**, the phenyl substituted probe **2** and lysate alone**.** Samples of the inputs and eluates from each immunoprecipitation were separated by SDS-PAGE and visualised by western blot

2. Biological methods

2.1 SDS-PAGE

Proteins were separated on a 12% acrylamide gel (resolving gel: 1.3 mL 1.5 M Tris pH 6.8, 1.5 mL 40% acrylamide/Bis-acrylamide (29:1) , 2 mL dH_2O, 50 µL 10% SDS, 50 µL 10% ammonium persulfate (APS), 5 µL Tetramethylethylenediamine (TEMED); stacking gel: 630 µL 0.5 M Tris pH 6.8, 300 µL acrylamide, 1.3 mL dH_2O, 25 µL 10% SDS, 25 µL 10% APS, 2.5 µL TEMED). Samples were prepared for separation by adding 2X reducing sample buffer (0.2 M Tris pH 6.8, 30% glycerol 0.4% β-mercaptoethanol, 9% SDS, bromophenol blue) followed by heating at 95 °C for 5 min. The proteins were loaded along with Fisher's EZ-Run^{TM} Pre-Stained Rec Protein Ladder. Separation was achieved at 150 V for 1-2 h and visualised either by western blotting or silver staining. All gels were imaged using Chemidoc XRS+ (Biorad, California USA) and Typhoon FLA9500 (GE Healthcare, Illinois USA).

2.2 Silver staining

Gels were treated with fixative (40% EtOH, 10% AcOH) at rt for 1 h or at 4 °C for 16 h. Gels were washed in 20% EtOH (2 x 10 min), then in dH_2O (2 x 10 min). Gels were sensitised in aq. $Na_2S_2O_3$ (0.02%) for 45 s and then immediately washed with dH_2O (2 x 1 min). The gel was incubated in a solution of $AgNO_3$ (12 mM) with formaldehyde (0.02%) at 4 °C for a

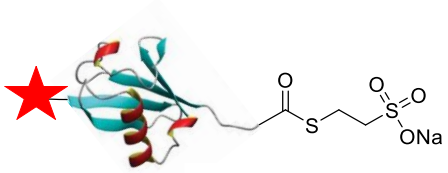
minimum of 20 min and up to 2 h. Following this, the gel was washed in dH_2O (2 x 30 s) and transferred to developer solution (3% K_2CO_3, 0.05% formaldehyde). Development was stopped using 5% AcOH.

2.3 Western Blotting

Proteins were transferred onto nitrocellulose membranes (GE Healthcare, Illinois USA) in blotting transfer buffer (25 mM Tris, 190 mM glycine, 20% MeOH) overnight at 15 V and 4 °C. The membrane was incubated in blocking solution (5% skimmed milk powder in PBST: 8 mM Na_2HPO_4, 150 mM NaCl, 2 mM KH_2PO_4, 3 mM KCl, 0.1% Tween 20, pH 7.4) for 1 h at rt or 16 h at 4 °C prior to immunoblotting. The primary mouse monoclonal anti-HA antibody (Biolegend, California USA) was diluted 1:2000 in blocking buffer and incubated with the membrane for 1 h at rt with gentle shaking. The membrane was washed with PBST (2 x 5 min) and PBS (2 x 5 min). The secondary antibody (Jackson ImmunoResearch, Cambridgeshire UK) was diluted in blocking buffer 1:4000, added to the membrane and incubated for 1 h at rt with gentle shaking. The membrane was washed with PBST (3 x 5 min), PBS (2 x 5 min) and dH_2O (1 x 5 min). Pierce ECL western blotting substrate (Thermofisher, Massachusetts USA) was used to visualise the chemiluminescence.

2.4 Synthesis of HA-tagged activity-based monoubiquitin probes

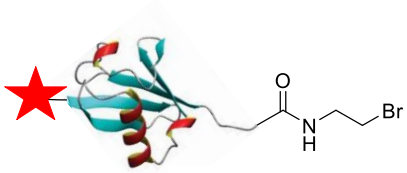
2.4.1 Expression and purification of HA-Ub_{75}-MeSNa



S1

The expression and purification of HA-Ub_{75}-MeSNa was carried out according to literature procedures.[5, 6] BL21 (DE3) cells transfected with a pTYB2 plasmid encoding for a HA-tagged ubiquitin75 fusion protein containing an intein domain and chitin-binding domain (HA-Ub_{75}-intein-CBD) were transferred from a glycerol stock into LB medium (8 mL) containing ampicillin (100 µg/mL) and grown for 18 h at 37 °C at 180 rpm. The cells were transferred into fresh LB medium (300 mL) containing ampicillin (100 µg/mL) and grown at 37 °C at 180 rpm until an OD_{600} of 0.6 to 0.9 was reached. IPTG was added at a final concentration of 0.4 mM and the bacteria were incubated at 18 °C for 16 h with vigorous shaking. The cells were centrifuged at 8000 rpm for 15 min. The resulting pellet was re-suspended in column buffer (20 mL, 50 mM HEPES pH 6.8, 100 mM NaOAc) and lysed *via* sonication. The lysate was centrifuged at 14000 rpm for 45 min. A column containing chitin resin (2.5 mL) (New England Biolabs) was equilibrated with column buffer (25 mL). The clarified supernatant was run over this column. The column was washed with column buffer (25 mL). After these washes, column buffer containing sodium 2-sulfanylethanesulfonate (MeSNa) (7.5 mL; 50 mM) was run through the column before incubation in this buffer for 18 h at 37 °C with gentle shaking. HA-Ub_{75}-MeSNa was eluted in column buffer (5 mL) before concentration by spinning at 14,000 rpm in Vivaspin 500 centrifugal concentrators (Sartorious, Göttingen Germany). HA-Ub_{75}-MeSNa was desalted using a NAP-5 column (GE Healthcare, Illinois USA) and eluted in column buffer according to manufacturer's instructions. The sample was concentrated again at 14,000 rpm using Vivaspin centrifugal concentrators and the protein concentration was measured on a nanodrop (4.8 mg/mL, 100 µL).

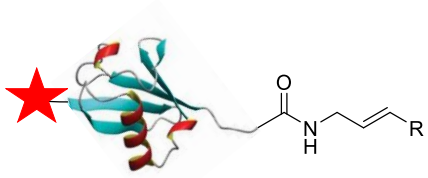
2.4.2 Coupling HA-UB$_{75}$-MeSNa to bromide warhead



S2

HA-Ub$_{75}$CH$_2$CH$_2$Br was synthesised using literature procedures.[6] 2-bromoethylamine·HBr (31 mg, 0.15 mmol) was dissolved in column buffer (200 µL) and the pH of the solution was adjusted to pH 8.0 by the addition of aq. NaOH (1 M). HA-Ub$_{75}$-MeSNa in column buffer (2.2 mg/mL, 100 µL) was added to this solution and it was shaken gently for 90 min at rt. The reaction mixture was desalted using a NAP-5 column according to manufacturer's instructions, eluted in column buffer and concentrated by centrifuging at 14,000 rpm in a Vivaspin centrifugal concentrator. The protein concentration was measured on a nanodrop (1.5 mg/mL, 100 µL).

2.4.3 Coupling HA-Ub$_{75}$-MeSNa to alkene warheads



Probe 1: R = H
Probe 2: R = Ph

N-Hydroxysuccinimide (0.2 M, 45 µL) and Tris base (100 mM, 10 µL, pH 7.5) were added to HA-Ub$_{75}$-MeSNa in column buffer (1.2 mg/mL, 500 µL) and incubated for 10 min at rt. Allylamine (23 µL, 0.3 mmol) or cinnamylamine (40 mg, 0.3 mmol) was added to a solution of MeCN-H$_2$O (1:1, 56 µL). This solution was added to the reaction mixture and the pH was adjusted to 9.0. The reaction was incubated for 18 h at 37 °C with gentle shaking. After this time, the reaction mixture was desalted using a NAP-5 column according to manufacturer's instructions and concentrated in a Vivaspin centrifugal concentrator at 14,000 rpm. The protein concentration was measured on a nanodrop, probe **1** = (3.4 mg/mL, 100 µL); probe **2** = (5.6 mg/mL, 100 µL).

2.5 *In vitro* DUB labelling

2.5.1 HEK293T cell lysate preparation

A HEK293T cell pellet was lysed using glass beads. To a 100 µL cell pellet, 100 µL of glass beads were added. Homogenisation buffer (200 µL; 50 mM Tris pH 7.4, 5 mM $MgCl_2$, 250 mM sucrose, 1 mM DTT or 1 mM TCEP) was added. The mixture was vortexed for 20 s before being placed on ice for 90 s. This sequence was repeated 20 times. Cell debris and glass beads were pelleted by centrifuging at 14,000 rpm for 5 min. The resulting supernatant was aspirated off. The protein concentration of the clarified extract was measured by nanodrop (19.9 mg/mL, 200 µL)

2.5.2 *In vitro* Ub$_{75}$CH$_2$CH$_2$Br probe labelling

HA-Ub$_{75}$-Br probe **S2** (0.75 µL, 1.5 mg/mL in column buffer) was incubated with HEK293T cell lysate (2.5 µL, 19.9 mg/mL in homogenisation buffer). The final volume of the labelling was

adjusted to 30 µL with homogenisation buffer for the lysate labelling. Incubation was carried out for 90 min at 37 °C with gentle shaking. Upon completion, 2X reducing sample buffer (15 µL) was added and the proteins were heated to 95 °C for 5 min. The samples were separated using a 12% SDS-PAGE and visualised using silver staining or western blotting.

2.5.3 Optimised *In vitro* thiol-ene labelling with alkene probes

The relevant alkene probe (1 - 4 µg) was incubated with HEK293T cell lysate (2.5 µL, 19.9 mg/mL in homogenisation buffer) or OTUB1 (2 µg). The final volume of the labelling was adjusted to 30 µL with homogenate buffer containing TCEP (1 mM) for the lysate labelling, or phosphate buffer (pH 8.0) containing TCEP (1 mM) for the recombinant enzyme labelling. The probes were pre-incubated with the DUBs for 90 min at 37 °C with gentle shaking before the addition of radical initiator 2,2-dimethoxy-2-phenyl-acetophenone (DPAP) (0.25 µM) and radical stabiliser 4'-Methoyacetophenone (MAP) (0.25 µM). The reaction mixture was degassed for 2 min with N_2 and exposed to UV light (365 nm) for 2 min. 2X reducing sample buffer (30 µL) was added and the samples were heated at 95 °C for 5 min. Proteins where visualised using silver staining and western blotting after being separated on a 12% SDS-PAGE.

2.5.4 In vitro thiol-ene labelling with alkene probes and denatured OTUB1

OTUB1 (2 µg) was denatured either by heating at 95 °C for 10 min or by the addition of SDS (0.5% final concentration). The final volume of the labelling was adjusted to 30 µL with phosphate buffer (pH 8.0) containing TCEP (1 mM). In this step SDS concentration was reduced fifteen-fold. The probes were pre-incubated with the DUBs for 90 min at 37 °C with gentle shaking before the addition of radical initiator DPAP (0.25 µM) and radical stabiliser MAP (0.25 µM). The reaction mixture was degassed for 2 min with N_2 and exposed to UV light (365 nm) for 2 min. 2X reducing sample buffer (30 µL) was added and the samples were heated at 95 °C for 5 min. Proteins where visualised using silver staining and western blotting after being separated on a 12% SDS-PAGE.

2.5.5 PR-619 pre-incubation assay

PR-619 was pre-incubated with HEK293T cell lysate (2.5 µL, 19.9 mg/mL) on ice for 30 min at a range of concentrations. Probe **1** (0.3 µL, 3.4 mg/mL in column buffer) was added giving the labelling a final volume of 30 µL. The reaction mixture was incubated for a further 90 min before addition of DPAP (0.25 µM) and MAP (0.25 µM) and degassing for 2 min with N_2. The mixture was exposed to UV light (365 nm) for 2 min. 2X reducing sample buffer (30 µL) was added and the samples were heated at 95 °C for 5 min.

2.5.6 PR-619 equilibrium disruption assay

Probe **1** (0.3 µL, 3.4 mg/mL in column buffer) or HA-Ub_{75}-Br probe **S2** (0.75 µL, 1.5 mg/mL in column buffer) was incubated with HEK293T cell lysate (2.5 µL, 19.9 mg/mL) at 37 °C for 60 min. PR-619 was added at a range of concentrations and the mixture was incubated for a further 30 min at 37 °C. DPAP (0.25 µM) and MAP (0.25 µM) were added and the mixture was degassing for 2 min with N_2. The mixture was exposed to UV light (365 nm) for 2 min. 2X reducing sample buffer (30 µL) was added and the samples were heated at 95 °C for 5 min.

2.6 Immunoprecipitation (IP)

The relevant alkene probe (5 µg) was pre-incubated with HEK293T cell lysate (12.5 µL, 19.9 mg/mL in homogenate buffer) in NET buffer (136 µL; 50 mM Tris pH 7.5, 5 mM EDTA, 150 mM NaCl, 0.5% NP-40) containing TCEP (1 mM) for 90 min at 37 °C. DPAP (0.25 µM) and MAP (0.25 µM) were added and the solution was degassed with N_2 for 2 min. The solution was

exposed to UV light (365 nm) for 2 min. SDS solution (10% in dH_2O, 7.5 µL) was added to the reaction before vortexing for 30 s and sonication for 2 min. The mixture was diluted with homogenate buffer (1500 µL). EZview™ Red Anti-HA Affinity Gel (100 µL of 50% slurry) was equilibrated by adding NET buffer (750 µL), gently inverting and centrifuging at 9000 rpm. The supernatant was aspirated, and the equilibration step was repeated. The lysate was added to the equilibrated beads and incubated at 4 °C for 90 min with rolling. The mixture was centrifuged at 9000 rpm for 30 s and the supernatant was aspirated. NET buffer (750 µL) was added to the beads which were inverted until the beads were fully resuspended before being centrifuged at 9000 rpm for 30 s. This washing step was repeated four times. After the final wash glycine buffer (250 µL, 150 mM, pH 2.5) was added to the beads. The solution was inverted until the beads were resuspended and then left on ice for 1 min. The solution was centrifuged at 9000 rpm for 30 s. The resulting supernatant was aspirated, and this elution step was repeated. 1X reducing sample buffer (250 µL) was added to the beads which were heated at 95 °C for 5 min. A small % of the samples were separated by 12 % SDS-PAGE and visualised by western blotting. The remainder of the samples was subject to tryptic digest using the FASP protocol, desalted by zip tipping and analysed by LC-MS/MS using an Orbitrap.

2.7 Mass spectrometry

2.7.1 $CHCl_3$/MeOH extraction

Probe samples were concentrated using a $CHCl_3$/MeOH extraction prior to an in-solution digest to identify the C-terminal peptide. MeOH (600 µL) and $CHCl_3$ (150 µL) were added to a sample of protein (200 µL) and the solution was vortexed for 20 s. dH_2O (450 µL) was added and the sample was vortexed for a further 20 s. The sample was centrifuged at 14,000 rpm for 2 min. The upper layer was aspirated off and discarded. The sample was diluted with MeOH (450 µL), vortexed for 20 s and centrifuged at 14,000 rpm for 1 min. The supernatant was aspirated and discarded. The pellet was prepared for an in-solution digestion.

2.7.2 In-solution digest following $CHCl_3$/MeOH extraction

The protein pellet obtained using a $CHCl_3$/MeOH extraction was diluted in urea buffer (50 µL; 6 M urea, 33 mM Tris pH 7.8) and dissolved by vortexing for 20 s and sonicating for 2 min. The sample was diluted with dH_2O (250 µL), vortexed for 20 s and sonicated for a further 2 min. Elastase was added in a 1:15 dilution relative to the protein concentration. The digest was carried out at 37 °C with gentle shaking for 16 h. Samples were prepared for MS analysis by zip-tipping analysed by captive spray ionisation mass spectrometry

2.7.3 In-gel digest

Samples were separated by SDS-PAGE and visualised by silver staining. Bands of interest were excised, cut into small pieces and incubated for 18 h in wash solution (200 µL; 50% MeOH, 45% dH_2O, 5% formic acid). The wash solution was aspirated, and fresh wash solution was added. The samples were incubated for a further 2 h at rt. The wash solution was removed, and the gel pieces were dehydrated for 5 min in MeCN (2 x 200 µL). DTT buffer (30 µL; 100 mM NH_4HCO_3, 10 mM DTT) was added to the gel pieces and they were incubated for 30 min at rt. The DTT buffer was removed and iodoacetamide solution (30 µL, 50 mM) was added. The samples were incubated for a further 30 min. After the removal of the iodoacetamide solution the gel pieces were dehydrated for 5 min in MeCN (200 µL). Rehydration was performed in NH_3HCO_3 solution (200 µL, 100 mM). Dehydration and rehydration steps were repeated. Trypsin stock was diluted in NH_3HCO_3 solution and this 1X stock (30 µL, 20 µg/mL) was added to the dehydrated gel pieces. The solution was incubated

on ice for 10 min with gentle mixing. Following this incubation step, NH_3HCO_3 solution (5 µL, 50 mM) was added to the mixture and it was incubated for 18 h at 37 °C with gentle shaking. After this incubation, NH_3HCO_3 solution (50 µL, 50 mM) was added. The gel pieces were incubated in this mixture for 10 min with occasional vortexing. The supernatant was transferred to a fresh microcentrifuge tube. Extraction buffer 1 (50 µL; 50% MeCN, 45% dH_2O, 5% formic acid) was added to the gel pieces. The pieces were incubated for 10 min in this buffer with occasional vortexing. The supernatant was then added to the collection tube and the gel pieces were incubated for a further 10 min in extraction buffer 2 with periodic vortexing (85% MeCN, 10% dH_2O, 5% formic acid). The supernatant was again added to the collection tube. For bigger protein bands an additional extraction with extraction buffer 2 was performed. The combined supernatants were dried in a vacuum centrifuge, resuspended in buffer A (20 µL; 98% dH_2O, 2% MeCN, 0.1 % formic acid) and analysed by captive spray ionisation mass spectrometry.

2.7.4 Filter Aided Sample Preparation (FASP)

FASP[7] was carried out using Vivaspin 500 centrifugal concentrators (10,000 MWCO). The concentrator was conditioned with 50 µL dH_2O. It was spun at 11800 rpm for 2 min. The protein solution to be digested was transferred to the filter and centrifuged at 13000 rpm until concentrated to a maximum of 25 µL. UA buffer (200 µL, 8 M urea in 0.1 M Tris/HCl pH 8.5) was added to the filter and centrifuged at 11800 rpm for 15 min. This step was repeated twice. DTT solution (100 µL, 10 mM in UA buffer) was added to the concentrator and vortexed for 5 s. It was centrifuged at 11800 rpm for 15 min. IAA solution (100 µL, 50 mM) was added and the solution was vortexed for 5 s and then centrifuged again at 11800 rpm for 15 min. Washes were performed using UA buffer (3 x 100 µL) followed by NH_4HCO_3 solution (3 x 100 µL, 50 mM). After the final wash, trypsin solution (200 µL, 50 mM NH_4HCO_3 solution, 1:50 enzyme:protein) was added and the concentrator was incubated overnight at 37 °C. The concentrator was centrifuged at 11800 rpm for 15 min. NaCl solution (50 µL, 0.5 M) was added and it was centrifuged at 11800 rpm until all the of the solution had passed the filter. Samples were de-salted by zip-tipping and analysed by LC-MS/MS.

2.7.5 Zip-tip purification

A zip-tip (Merk Millipore, Massachusetts USA) was equilibrated by aspirating and dispensing buffer B (for peptides: 80% MeCN, 20% H_2O, 0.1% TFA; for full proteins; 65% MeCN, 35% H_2O, 0.1% TFA) four times and further four times with buffer A (2% MeCN, 98% H_2O, 0.1% TFA). The protein sample was aspirated across the tip ten times. Buffer A was used to wash the sample by aspirating and dispensing four times. The protein or peptides were then eluted in buffer B (2 x 10 µL) and dried using a vacuum centrifuge. The sample was analysed by MALDI or LC-MS/MS.

2.7.6 MALDI-TOF MS

MALDI-TOF analysis was carried out on a BRUKER Ultraflextreme MALDI-TOF/TOF mass spectrometer. The matrix used was a saturated solution of HCCA (α-Cyano-4-hydroxycinnamic acid) in TA 85% (85% ACN with 0.1% TFA), and the calibrant was prepared in the same matrix. The matrix (1 µL) was mixed with the sample (1 µL) and 1µL of this mixture was deposited onto a ground steel MALDI target plate and allowed to dry in air. Mass spectra were recorded in positive reflection mode.

2.7.7 Orbitrap mass spectrometry

Protein digests were redissolved in 0.1% TFA (30 µL per sample) by agitation (1200 rpm, 15 min) and sonication in an ultrasonic water bath (10 min). This was followed by centrifugation

(14,000 rpm, 5 °C, 10 min) and transfer to MS sample vials. LC-MS/MS analysis was carried out in technical duplicates (4.0 µL per injection) and separation was performed using an Ultimate 3000 RSLC nano liquid chromatography system (Thermo Scientific) coupled to a Orbitrap Velos mass spectrometer (Thermo Scientific) *via* an Easy-Spray nano-electrospray source (Thermo Scientific). Samples were injected and loaded onto a trap column (Acclaim PepMap 100 C18, 100 µm × 2 cm) for desalting and concentration at 8 µL/min in 2% acetonitrile, 0.1% TFA. Peptides were then eluted on-line to an analytical column (Acclaim Pepmap RSLC C18, 75 µm × 50 cm) at a flow rate of 250 nL/min. Peptides were separated using a 120 min gradient, 4-25% of buffer A for 90 min followed by 25-45% buffer B for another 30 min (buffer A: 5% DMSO, 0.1% FA; buffer B: 75% acetonitrile, 5% DMSO, 0.1% FA) and subsequent column conditioning and equilibration. Eluted peptides were analysed by the mass spectrometer operating in positive polarity using a data-dependent acquisition mode. Ions for fragmentation were determined from an initial MS1 survey scan at 30,000 resolution, followed by CID (Collision-Induced Dissociation) of the top 10 most abundant ions. MS1 and MS2 scan AGC targets were set to 1^6 and 3^4 for maximum injection times of 500 ms and 100 ms respectively. A survey scan m/z range of 350 – 1500 was used, normalised collision energy set to 35%, charge state screening enabled with +1 charge state rejected and minimal fragmentation trigger signal threshold of 500 counts. Data was processed using the MaxQuant [8] software platform (v1.6.7.0), with database searches carried out by the in-built Andromeda search engine against the Swissprot *H.sapiens* database (version 20180104, number of entries: 20,244). A reverse decoy database approach was used at a 1% false discovery rate (FDR) for peptide spectrum matches. Search parameters included: maximum missed cleavages set to 3, fixed modification of cysteine carbamidomethylation and variable modifications of methionine oxidation, asparagine deamidiation and protein N-terminal acetylation. Label-free quantification was enabled with an LFQ minimum ratio count of 1.

2.7.8 Captive spray ionisation mass spectrometry

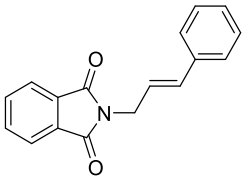
Captive spray ionisation was performed using a Thermo Scientific UltiMate 3000RSLCnano LC (Waltham, MA USA) equipped with an Acclaim PepMap C18 (2 µm, 0.075 mm x 150 mm) column. For each injection, 5 µL of a (1 µg/µL) digested peptide was loaded onto a Nano Trap Column (100 µm I.D. x 2 cm, packed with Acclaim PepMap100 C18) at 10 µL/min with 95% water/ 5% acetonitrile/ 0.1% formic acid for 3 min. Trapped peptides were eluted onto the analytical column using a multi-step gradient with a flow rate of 0.3 µL/min. The gradient utilised two mobile phase solutions: A, water/0.1% formic acid and B, acetonitrile: 0 min, A (98%), B (2%); 3 min, A (98%), B (2%); 63 min A (65%), B (35%); 64 min A (5%), B (95%); 66 min A (5%), B (95%); 67 min A (98%), B (2%); 75 min A (98%), B (2%). Peptide digest were analysed on a Bruker compact Qq-TOF mass spectrometer via CaptiveSpray nanoBooster (Bremen Germany). Precursor ions were scanned from 150 m/z to 2200 m/z at 2 Hz with a cycle time of 3.0 seconds, with fixed windows excluded (20-350, 1221-1225, 2200-40000). Smart Exclusion was used to ensure only chromatographic peaks were selected as precursors. Active Exclusion enabled the analysis of less-abundant ions to be analysed and not excluded from precursor selection. Data acquired on the Bruker compact was converted to mzXML format and searched against a custom database containing the probe sequence inserted into a uniprot database with taxonomy restricted to human on PeptideShaker. [9]

3. General chemical methods

^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 MHz or 600 MHz system spectrometers. Spectra were recorded in DMSO-d_6 or $CDCl_3$ relative to residual DMSO (δ =

2.50 ppm) or CHCl3 (δ = 7.26 ppm). Chemical shifts are reported in parts per million (ppm), coupling constants are reported in Hertz (Hz) and are accurate to 0.2 Hz. NMR spectra were assigned using HSQC and HMBC experiments. Mass spectrometry measurements were carried out on a Bruker ESI or APCI HRMS. Melting points were measured using a Griffin melting points apparatus and are uncorrected. Infrared (IR) spectra were obtained on a Perkin Elmer spectrophotometer. Flash column chromatography was carried out using silica gel, particle size 0.04-0.063 mm. TLC analysis was performed on precoated $60F_{254}$ slides and visualised by UV irradiation, potassium permanganate stain (3 g $KMnO_4$, 20 g K_2CO_3, 300 mL dH_2O) and ninhydrin stain (1.5 g ninhydrin, 5 mL, AcOH, 500 mL EtOH 95%). All solvents were obtained from commercial sources and used as received. Petroleum ether refers to the fraction of petroleum ether that boils at 40-60 °C.

3.1 Synthesis of *(E)*-1-phenyl-3-phthalimido-2-propene



S3

Cinnamyl bromide (500 mg, 2.54 mmol) and potassium phthalimide (729 mg, 3.94 mmol) were dissolved in dry DMF (10 mL) under argon. The reaction mixture was stirred at rt for 3 h. TLC analysis (petroleum ether) showed complete consumption of cinnamyl bromide (R_f = 0.6) and formation of the product (R_f = 0.1) after this time. The solution was diluted with Et_2O (40 mL) and brine (30 mL) and the white precipitate formed was collected by vacuum filtration. The aqueous layer was extracted with Et_2O (2 x 30 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated to afford the crude product as a yellow solid. This was combined with the precipitated product and recrystallised from toluene to afford the product **S3** as colourless crystals (411 mg, 62%); mp 152 – 154 °C (toluene). Lit. [10] 154 °C – 155 °C.

ν_{max}/cm^{-1} (neat) 3461 (C-H_{ar}), 3102 (C-H_{ar}), 3083 (C-H_{ar}), 3026 (C-H), 2915 (C-H), 1769 (C=O), 1701 (C=O), 1426 (C=C), 1394, 1321, 1106, 969, 952, 724.

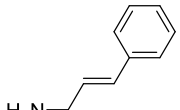
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.86 (dd, *J* = 2.9 Hz, *J* = 5.5 Hz, 2H, CH_{Phth}), 7.72 (dd, *J* = 2.9 Hz, *J* = 5.5 Hz , 2H, CH_{Phth}), 7.37 – 7.32 (m, 2H, CH_{Ph}), 7.31 – 7.27 (m, 2H, CH_{Ph}), 7.24 – 7.20 (m, 1H, CH_{Ph}), 6.66 (d, *J* = 16.1 Hz, 1H, CH=C<u>H</u>-Ph), 6.28 (dt, *J* = 6.7 Hz, *J* = 16.0 Hz, 1H, C<u>H</u>=CH-Ph), 4.45 (d, *J* = 6.7 Hz, 2H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.2 (C=O), 136.2 (qC_{Ph}), 134.0 (qC_{Phth}), 133.7 (CH_{Phth}), 132.2 (<u>C</u>H=CH-Ph), 128.5 (CH_{Ph}), 127.9 (CH_{Ph}), 126.5 (CH_{Ph}), 123.3 (CH_{Phth}), 122.8 (CH=<u>C</u>H-Ph), 39.7 (CH_2) ppm.

HRMS ($APCI^+$): *m/z* calc. 264.1025 $[M+H]^+$, found 264.1014

The spectroscopic data is in agreement with those reported in the literature.[11]

3.2 Synthesis of *(E)*-3-phenyl-prop-2-en-1-amine



S4

(E)-1-phenyl-3-phthalimido-2-propene **S3** (700 mg, 2.66 mmol) was dissolved in MeOH (12 mL). Hydrazine hydrate solution (80%, 150 µL, 2.95 mmol) was added dropwise and the reaction was stirred at rt for 2 h. TLC analysis after this time showed the complete consumption of the starting material (petroleum ether-EtOAc, 3:1; R_f = 0.8) and formation of the product **S4** (H_2O-IPA-EtOAc, 1:2:2; R_f = 0.2). The reaction was cooled to 4 °C resulting in the formation of a white precipitate. The white precipitate was isolated by vacuum filtration and washed with MeOH (3 x 10 mL). The filtrate was concentrated under reduced pressure and the residue was dissolved in DCM (20 mL) and aq. KOH (20 mL). The aqueous layer was extracted with DCM (3 x 20 mL) and the combined organic layers were concentrated to afford the product **S4** as a yellow oil (228 mg, 65%).

ν_{max}/cm^{-1} (neat) 3676 (NH_2), 3261 (C-H_{ar}), 2988 (C-H), 2902 (C-H), 1570 (C=C), 1447, 1403, 1315, 1274, 1067, 1057, 964, 739, 689.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.37 (d, *J* = 7.4 Hz, 2H, CH_{Ph}), 7.33-7.27 (m, 2H, CH_{Ph}), 7.25 – 7.15 (m, 1H, CH_{Ph}), 6.50 (d, *J* = 15.9 Hz, 1H, CH=C<u>H</u>-Ph), 6.32 (dt, *J* = 5.9 Hz, *J* = 15.9 Hz, 1H, C<u>H</u>=CH-Ph), 3.48 (d, *J* = 5.9 Hz, 2H, CH_2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ = 137.2 (qC), 131.1 (CH_{Ph}), 129.6 (CH_{Ph}), 128.5 (CH_{Ph}), 127.3 (CH=<u>C</u>H-Ph), 125.8 (<u>C</u>H=CH-Ph), 44.3 (CH_2) ppm.

HRMS (ESI+): *m/z* calc. 136.1121 $[M+H]^+$, found: 136.1126

The spectroscopic data is in agreement with those reported in the literature.[12]

References:

[1] M. Altun, T. S. Walter, H. B. Kramer, P. Herr, A. Iphöfer, J. Boström, Y. David, A. Komsany, N. Ternette, A. Navon, D. I. Stuart, J. Ren, B. M. Kessler, *PLoS One* **2015**, *10*, e0115344.

[2] A. Ernst, G. Avvakumov, J. Tong, Y. Fan, Y. Zhao, P. Alberts, A. Persaud, J. R. Walker, A. M. Neculai, D. Neculai, A. Vorobyov, P. Garg, L. Beatty, P. K. Chan, Y. C. Juang, M. C. Landry, C. Yeh, E. Zeqiraj, K. Karamboulas, A. Allali-Hassani, M. Vedadi, M. Tyers, J. Moffat, F. Sicheri, L. Pelletier, D. Durocher, B. Raught, D. Rotin, J. Yang, M. F. Moran, S. Dhe-Paganon, S. S. Sidhu, *Science* **2013**, *339*, 590-595.

[3] S. Misaghi, P. J. Galardy, W. J. Meester, H. Ovaa, H. L. Ploegh, R. Gaudet, *J Biol Chem* **2005**, *280*, 1512-1520.

[4] W. Tian, C. Chen, X. Lei, J. Zhao, J. Liang, *Nucleic Acids Res* **2018**, *46*, W363-W367.

[5] S. Chong, F. B. Mersha, D. G. Comb, M. E. Scott, D. Landry, L. M. Vence, F. B. Perler, J. Benner, R. B. Kucera, C. A. Hirvonen, J. J. Pelletier, H. Paulus, M. Q. Xu, *Gene* **1997**, *192*, 271-281.

[6] A. Borodovsky, H. Ovaa, N. Kolli, T. Gan-Erdene, K. D. Wilkinson, H. L. Ploegh, B. M. Kessler, *Chem Biol* **2002**, *9*, 1149-1159.

[7] L. L. Manza, S. L. Stamer, A. J. Ham, S. G. Codreanu, D. C. Liebler, *Proteomics* **2005**, *5*, 1742-1745.

[8] J. Cox, M. Mann, *Nat Biotechnol* **2008**, *26*, 1367-1372.

[9] M. Vaudel, J. M. Burkhart, R. P. Zahedi, E. Oveland, F. S. Berven, A. Sickmann, L. Martens, H. Barsnes, *Nat Biotechnol* **2015**, *33*, 22-24.

[10] T. Jensen, H. Pedersen, B. Bang-Andersen, R. Madsen, M. Jørgensen, *Angewandte Chemie International Edition* **2008**, *47*, 888-890.

[11] K. M. Lambert, J. M. Bobbitt, S. A. Eldirany, L. E. Kissane, R. K. Sheridan, Z. D. Stempel, F. H. Sternberg, W. F. Bailey, *Chemistry – A European Journal* **2016**, *22*, 5156-5159.

[12] M. Hari Babu, G. Ranjith Kumar, R. Kant, M. Sridhar Reddy, *Chemical Communications* **2017**, *53*, 3894-3897.